Expression of Apoptosis Signal-Regulating Kinase 1 (ASK1) in Mouse Spinal Cord Under Chronic Mechanical Compression

**Introduction**

Chronic spinal cord compression due to spondylosis, disc herniation, or ossification of the posterior longitudinal ligament (OPLL) is known to induce degenerative spinal cord changes, such as myelin destruction and loss of axons in the white matter and neurons in the gray matter, which can result in profound and irreversible motor paresis. However, the mechanism of these destructive changes has not been clarified at the molecular biological level.

Recently, we demonstrated induction of apoptosis in chronically compressed spinal cord. TUNEL-positive neurons and oligodendrocytes were detected in chronically compressed cervical cords of an autopsied patient and mice exhibiting chronic spinal cord compression, but not in the uncompressed spinal cord. TUNEL-positive spinal cord cells were most apparent in severely degenerative areas, such as the anterior, lateral, and posterior columns and central gray matter at the site of compression, and the ascending tracts rostral to and the descending tracts caudal to the site of compression. However, the molecular mechanisms of apoptosis in chronically compressed spinal cord remain unclear.

We recently demonstrated in acute spinal cord injury that the Apoptosis Signal-regulating Kinase 1 (ASK1) -c-Jun N-terminal kinase (JNK) -p38 pathways, which include members of the mitogen activated protein kinase (MAPK) family, transmit apoptosis signals to neurons and glia. ASK1 is activated by various external stressors or by cytokines. Activated ASK1 promotes activation of JNK and p38 resulting in apoptosis. To examine the role of stress-activated MAPK pathways including ASK1 in transmission of apoptosis signals in chronically compressed spinal cord, we examined the expression and localization of activated ASK1, activated JNK, activated p38 and activated caspase-3 immunohistologically using the tiptoe-walking Yoshimura (twy) mouse, an animal model of progressive cervical cord compression.

**Materials and Methods**

Adult twy mice (Central Institute for Experimental Animals, Kawasaki, Japan), 6 months old and weighing 25 to 31 g, which exhibited spinal cord compression were used in this study. The twy mouse is produced by brother-sister mating of Institute of Cancer Research (ICR) mice, and is considered a mutant of autosomal inheritance, without congenital neural abnormalities.

Immunohistochemistry was performed with using affinity-purified polyclonal antibody against activated caspase-3, phospho-specific JNK, phospho-specific p38, and activated ASK1 antibodies on spinal cord sections of ICR mice and twy mice aged 1 month and 6 months.

**Result**

Expression of ASK1

In control mice, no cells expressed activated ASK1. In twy mice aged 6 months, activated ASK1-positive cells were observed in gray and white matter at the site of compression. The number of cells with expression of activated ASK1 gradually decreased with distance from the site of compression.

Expression of activated JNK and activated p38

In control mice, only a few cells expressed activated JNK or activated p38. In twy mice aged 6 months, a large number of cells with expression of activated JNK or activated p38 were found in gray and white matter. Expression of activated JNK and activated p38 was observed at both the site of compression and adjacent regions, but was most apparent at the site of compression.

Expression of Caspase-3

In control mice, no cells expressed caspase-3. In twy mice aged 6 months, activated caspase-3-positive cells were seen in the gray and white matter at the site of compression and adjacent regions. The number of activated caspase-3-positive cells gradually decreased with distance from the site of compression.

**Discussion**

We recently demonstrated that apoptosis occurred in both neuron and glia in spinal cord under chronic mechanical compression. The apoptosis associated with chronic spinal cord compression may participate in profounding and irreversible motor paresis caused by destructive pathological spinal cord changes. In the present study, we should that examined the expression of ASK1, activated JNK, activated p38 and caspase-3 immunohistologically in the TWY mouse, an animal model of progressive cervical cord compression, since the ASK1–JNK and -p38 signaling cascades participate in the signaling pathway leading to apoptosis in neural tissue and neuronal culture.

These investigations suggest the possible involvement of the ASK1–JNK and -p38 signaling cascade may participate in the transmission of apoptosis signals in mouse spinal cord under chronic compression.